Reviewer’s report

Title: Enhancing SHP-1 expression by 5-Azacytidine inhibits STAT3 activation and confers sensitivity in Lestaurtinib (CEP-701) resistant FLT3-ITD positive Acute Myeloid Leukemia

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Reviewer: Friedrich Stölzel

Reviewer’s report:

The manuscript “Enhancing SHP-1 expression by 5-Azacytidine inhibits STAT3 activation and confers sensitivity in Lestaurtinib (CEP-701) resistant FLT3-ITD positive Acute Myeloid Leukemia” by Al-Jamal et al. describes the generation of a TKI (Lestaurtinib) resistant AML cell line MV4-11. Furthermore, the clinically important issue of how to overcome this resistance is addressed. In short summary, the authors conclude that the re-expression/restoration of SHP-1 expression by treatment with the hypomethylating agent 5-Aza (re-) induces sensitivity towards AML cell-line cells which were previously Lestaurtinib resistant.

While this manuscript tackles a clinically important key point, there are still major limitations as to how the data are presented and interpreted.

The authors ask a question which has been valid for many years now (resistance to TKIs in AML) and try to find a suitable target. Since 5-Aza has been approved for treatment for patients with MDS and AML with low blast counts, this is an interesting approach and moreover the authors try to find a mechanistic solution which could pave the way for more research which could eventually lead to better treatment options for AML patients.

I recommend to improve the manuscript on the following major compulsory revisions for publication:

1) The authors describe their hypothesis of SHP-1 expression being responsible for the mechanism of action – however, they perform gene expression microarray experiments to see whether this hypothesis were correct. It is unclear why to perform gene expression microarrays on thousands of genes when only this gene (and con- or discordant regulators of this gene) was/were suspected for the mechanism of action. Simple RT-PCRs would've been enough to prove the hypothesis. It seems to me that the experiments were performed first and then the hypothesis was born which makes more sense the way the story (the manuscript) is being told currently. Either clarify or re-write the background/introduction part.

2) Although the question asked is – from a clinically standpoint – important and scientifically well considered, the English-writing of the manuscript needs to be improved. Please either have the manuscript edited by a native speaker or by a commercially available English editing service.
3) Regarding the scientific data and the way those data are presented:

a. The figures are poorly edited—the message is clear but in order to improve the way the readership can follow they need to be improved with regard to style. They need to be merged in order to have less figures and the style needs to be homogenized—please see other published data and how these figures are presented.

b. Figures 1 A – C (although I know that from A to C they are read from left to right, I still need to guess since they are not titled adequately): why is there only one SD shown at 100 nM CEP-701 whereas in A and B 3 to 4 SDs are shown? Were other concentration measurements not performed? If yes, why? If not please do so.

c. It is not described (or it was not mentioned clear enough) whether the baseline proliferation in MV4-11 R-cep cells was also lower? This needs to be shown in order to be in line with the story.

d. Regarding the MTS on the cell line: what happens to MV4-11 (CEP-701 naïve) when being exposed to 5’Aza? This would be interesting to know.

e. The Western Blots have poor quality and the procedure has not been described in the M&M section. Please include in this section and perform blots so that the reader can see that this is from one gel and not from 21 different Western experiments. I would accept more Western blots with corresponding b-actin controls in favour of the 21 different pieces shown now.

4) Last but not least: 5-Aza is a hypomethylating agent and some people call it a “dirty drug”. With its hypomethylating activity my first guess would be that one will see a decreased SHP-1 methylation in these cells after 5-Aza exposure which is why the results do not surprise me. However, the authors conclude that the hypomethylation of exactly this gene (or its CpG islands) is responsible for the effect observed in resistant cells. This has not been proven from a scientific point of view—the authors should either specifically hypomethylate this region and then analyze whether they observe the same effects which is in my opinion not possible. Therefore, in order to be able to state what is written in this manuscript a siRNA-mediated approach is necessary. This could either be lentiviral or as transient si-SHP-1 transfection (substituting for the 5’-Aza effect) and then re-analyze restoration of sensitivity towards Lestaurtinib. The way it is performed now it could be any protein/gene responsible which was differentially regulated as per gene expression data. However, currently there is still no proof for the hypothesis of the authors that SHP-1 is the culprit.

5) Please also provide complete microarray data (also as supplement possible) for access for other researchers in the field as per consortium agreements.

Minor revisions:

1) Introduction: what is 5-Aza2dc—please clarify?
2) Results: generally, the readability needs to be improved since the results described go back and forth from Figure 1 to 2 and back to 1 and 2. This needs
to be edited for the readership.

3) Results: when describing gene expression please write genes in italics.

4) Discussion: first sentence: the resistance to TKIs remains “a” challenge not “the” challenge in treatment of AML patients.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests